

## What is claimed is:

- 1. A kit for the preparation of a cell preparation for tissue regeneration, the kit comprising, in one or more containers
  - a) a first component comprising an extracellular matrix or matrix material; and
  - b) a second component comprising one or more cell types that secrete a biologically active molecule, wherein the cell types are allogeneic, mitotically active or inactivated, and are selected from the group consisting of stromal, epithelial/organ specific and blood-derived cells.

10

5

- 2. The kit of claim 1, wherein the cell types are differentiated fibroblasts and keratinocytes.
- 3. The kit of claim 1, wherein the cell preparation is in the form of a paste.

- 4. The kit of claim 1, wherein the biologically active molecule is at least one angiogenic factor, at least one growth/cytokine factor, or a combination of at least one angiogenic factor and at least one growth/cytokine factor.
- 20 5. The kit of claim 1, wherein the extracellular matrix or matrix material is selected from the group consisting of fibrin, fibrin glue, fibrinogen, fibrin beads, and other synthetic polymer or polymer scaffolds or wound dressing materials.
- The kit of claim 1, wherein the cell types are mitotically inactivated by administration
   of mitomycin C or other chemically-based mitotic inhibitors, irradiation with γ-Rays,
   irradiation with X-Rays, or irradiation with UV light.
- 7. The kit of claim 1, wherein the cell types are immortalized using at least one gene/polypeptide selected from the group consisting of the 12S and 13S products of the adenovirus E1A genes, hTERT, SV40 small T antigen, SV40 large T antigen, papilloma viruses E6 and E7, the Epstein-Barr Virus (EBV), Epstein-Barr nuclear antigen-2 (EBNA2), human T-cell leukemia virus-1 (HTLV-1), HTLV-1 tax,



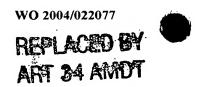
15

Herpesvirus saimiri (HVS), mutant p53, myc, c-jun, c-ras, c-Ha-ras, h-ras, v-src, c-fgr, myb, c-myc, n-myc, and Mdm2.

- 8. The kit of claim 1, wherein the cell types naturally secret one or more biologically active molecules.
  - 9. The kit of claim 1, wherein the cell types are genetically engineered to secrete an exogenous level of at least one angiogenic factor, at least one growth/cytokine factor, or a combination of at least one angiogenic factor and at least one growth/cytokine factor.
  - 10. The kit of claim 9, wherein the secretion of the at least one angiogenic factor, the at least one growth/cytokine factor, or the combination of at least one angiogenic growth and at least one growth/cytokine factor is controlled by gene switching.
  - 11. The kit of claim 9, wherein the at least one angiogenic factor, the at least one growth/cytokine factor, or the combination of at least one angiogenic growth and at least one growth/cytokine factor is constitutively secreted.
- 20 12. The kit of claim 1, wherein the first component comprises fibrinogen.
  - 13. The kit of claim 12, wherein the second component comprises from about  $1 \times 10^3$  cells/ $\mu$ l to about  $50 \times 10^3$  cells/ $\mu$ l.
- 25 14. The kit of claim 13, wherein the second component further comprises thrombin.
  - 15. The kit of claim 14, wherein the second component optionally further comprises a cryoprotectant.
- 30 16. The kit of claim 15, wherein the cryoprotectant is glycerol.

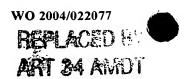


- 17. The kit of claim 1, wherein the first component comprises fibrinogen and the second component comprises from about 1 x 10<sup>3</sup> cells/μl to about 50 x 10<sup>3</sup> cells/μl and thrombin.
- 5 18. The kit of claim 17, wherein the second component optionally further comprises a cryoprotectant.
  - 19. The kit of claim 18, wherein the cryoprotectant is selected from the group consisting of a 10% glycerol solution, a 15% glycerol solution, and a 15% glycerol and 5% human serum albumin solution
  - 20. A method of using the kit of claim 1 to prepare a cell preparation for tissue regeneration, the method comprising
    - a) administering the first component to a wound site on a patient in need of treatment; and
    - b) combining the second component with the first component on the wound site wherein the combination of the first component and the second component forms a cell preparation suitable for tissue regeneration.
- 20 21. The method of claim 20, wherein the cell types are differentiated fibroblasts and keratinocytes.
  - 22. The method of claim 20, wherein the cell preparation is in the form of a paste.
- 25 23. The method of claim 20, wherein the biologically active molecule is at least one angiogenic factor, at least one growth/cytokine factor, or a combination of at least one angiogenic factor and at least one growth/cytokine factor.
- The method of claim 20, wherein the extracellular matrix or matrix material is
   selected from the group consisting of fibrin, fibrin glue, fibrinogen, fibrin beads, and other synthetic polymer or polymer scaffolds or wound dressing materials.



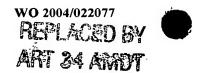
- 25. The method of claim 20, wherein the cell types are mitotically inactivated by administration of mitomycin C or other chemically-based mitotic inhibitors, irradiation with γ-Rays, irradiation with X-Rays, or irradiation with UV light.
- The method of claim 20, wherein the cell types are immortalized using at least one gene/polypeptide selected from the group consisting of the 12S and 13S products of the adenovirus E1A genes, hTERT, SV40 small T antigen, SV40 large T antigen, papilloma viruses E6 and E7, the Epstein-Barr Virus (EBV), Epstein-Barr nuclear antigen-2 (EBNA2), human T-cell leukemia virus-1 (HTLV-1), HTLV-1 tax,

  Herpesvirus saimiri (HVS), mutant p53, myc, c-jun, c-ras, c-Ha-ras, h-ras, v-src, c-fgr, myb, c-myc, n-myc, and Mdm2.
  - 27. The method of claim 20, wherein the cell types naturally secrete one or more biologically active molecules.
  - 28. The method of claim 20, wherein the cell types are genetically engineered to secrete an exogenous level of at least one angiogenic factor, at least one growth/cytokine factor, or a combination of at least one angiogenic factor and at least one growth/cytokine factor.
    - 29. The method of claim 28; wherein the secretion of the at least one angiogenic factor, the at least one growth/cytokine factor, or the combination of at least one angiogenic growth and at least one growth/cytokine factor is controlled by gene switching.
- 25 30. The method of claim 28; wherein the at least one angiogenic factor, the at least one growth/cytokine factor, or the combination of at least one angiogenic growth and at least one growth/cytokine factor is constitutively secreted.
- The method of claim 20, wherein the first component comprises fibrinogen and the
   second component comprises from about 1 x 10<sup>3</sup> cells/μl to about 50 x 10<sup>3</sup> cells/μl and thrombin.



25

- 32. The method of claim 31, wherein the second component optionally further comprises a cryoprotectant.
- 33. The method of claim 32, wherein the cryoprotectant is selected from the group consisting of a 10% glycerol solution, a 15% glycerol solution, and a 15% glycerol and 5% human serum albumin solution
  - 34. The method of claim 20, wherein the first and second components are topically administered to the wound site on the patient.
  - 35. The method of claim 20, wherein the first and second components are sprayed onto the wound site on the patient.
- 36. The method of claim 35, wherein the first and second components are combined on the wound site.
  - 37. The method of claim 35, wherein the first and second components are combined before reaching the wound site.
- 20 38. A method of administering a cell preparation for tissue regeneration to a wound site on a patient in need of treatment, the method comprising
  - a) providing a first component comprising an extracellular matrix or matrix material containing fibrinogen;
  - b) providing a second component comprising from about 1 x 10<sup>3</sup> cells/μl to about 50 x 10<sup>3</sup> cells/μl and thrombin, wherein the cells secrete one or more biologically active molecules, are allogeneic, mitotically inactivated, and selected from the group consisting of stromal, epithelia/organ specific, and blood-derived cells;
  - c) combining the first and second components to form a cell preparation suitable for tissue regeneration; and
  - d) administering the cell preparation to the wound site.



- 39. The method of claim 38, wherein the first and second components are topically applied to the wound site
- 40. The method of claim 38, wherein the first component is applied to the wound site before the second component is applied to the wound site.
  - 41. The method of claim 38, wherein the second component is applied to the wound site before the first component is applied to the wound site.
- 10 42. The method of claim 38, wherein the cells are differentiated or undifferentiated fibroblasts and keratinocytes.
  - 43. The method of claim 38, wherein the cell preparation is in the form of a paste.
- 15 44. The method of claim 38, wherein the biologically active molecule is at least one angiogenic factor, at least one growth/cytokine factor, or a combination of at least one angiogenic factor and at least one growth/cytokine factor.
- 45. The method of claim 38, wherein the extracellular matrix or matrix material is selected from the group consisting of fibrin, fibrin glue, fibrinogen, fibrin beads, and other synthetic polymer or polymer scaffolds or wound dressing materials.
  - 46. The method of claim 38, wherein the cell types are mitotically inactivated by administration of mitomycin C or other chemically-based mitotic inhibitors, irradiation with γ-Rays, irradiation with X-Rays, or irradiation with UV light.
  - 47. The method of claim 38, wherein the cell types are immortalized using at least one gene/polypeptide selected from the group consisting of the 12S and 13S products of the adenovirus *E1A* genes, hTERT, SV40 small T antigen, SV40 large T antigen, papilloma viruses E6 and E7, the Epstein-Barr Virus (EBV), Epstein-Barr nuclear antigen-2 (EBNA2), human T-cell leukemia virus-1 (HTLV-1), HTLV-1 tax,

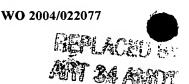


15

25

Herpesvirus saimiri (HVS), mutant p53, myc, c-jun, c-ras, c-Ha-ras, h-ras, v-src, c-fgr, myb, c-myc, n-myc, and Mdm2.

- 48. The method of claim 38, wherein the cell types naturally secret one or more biologically active molecules.
  - 49. The method of claim 38, wherein the cell types are genetically engineered to secrete an exogenous level of at least one angiogenic factor, at least one growth/cytokine factor, or a combination of at least one angiogenic factor and at least one growth/cytokine factor.
  - 50. The method of claim 49, wherein the secretion of the at least one angiogenic factor, the at least one growth/cytokine factor, or the combination of at least one angiogenic growth and at least one growth/cytokine factor is controlled by gene switching.
  - 51. The method of claim 49, wherein the at least one angiogenic factor, the at least one growth/cytokine factor, or the combination of at least one angiogenic growth and at least one growth/cytokine factor is constitutively secreted.
- 20 52. The method of claim 38, wherein the second component optionally further comprises a cryoprotectant.
  - 53. The method of claim 52, wherein the cryoprotectant is selected from the group consisting of a 10% glycerol solution, a 15% glycerol solution, and a 15% glycerol and 5% human serum albumin solution
  - 54. The method of claim 38, wherein the first and second components are sprayed on the wound site.
- The method of claim 54, wherein the first component is sprayed on the wound site before the second component is sprayed on the wound site.



- 56. The method of claim 54, wherein the sprayed first and second components are combined on the wound site.
- 57. The method of claim 54, wherein the sprayed first and second components are combined before reaching the wound site.
  - 58. A cell preparation for tissue regeneration comprising
    - a) a first component comprising an extracellular matrix or matrix material containing fibringen admixed with
- b) a second component comprising from about 1 x 10<sup>3</sup> cells/μl to about 50 x 10<sup>3</sup> cells/μl and thrombin to the wound site, wherein the cells secrete one or more biologically active molecules, are allogeneic, mitotically active or inactivated, and selected from the group consisting of stromal, epithelia/organ specific, and blood-derived cells.

- 59. The cell preparation of claim 58, wherein the cell types are differentiated fibroblasts and keratinocytes.
- 60. The cell preparation of claim 58, wherein the cell preparation is in the form of a paste.

- 61. The cell preparation of claim 58, wherein the biologically active molecule is at least one angiogenic factor, at least one growth/cytokine factor, or a combination of at least one angiogenic factor and at least one growth/cytokine factor.
- 25 62. The cell preparation of claim 58, wherein the cell types are mitotically inactivated by administration of mitomycin C or other chemically-based mitotic inhibitors, irradiation with γ-Rays, irradiation with X-Rays, or irradiation with UV light.
- one gene/polypeptide selected from the group consisting of the 12S and 13S products of the adenovirus *E1A* genes, hTERT, SV40 small T antigen, SV40 large T antigen, papilloma viruses E6 and E7, the Epstein-Barr Virus (EBV), Epstein-Barr nuclear



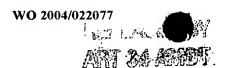
15

20

30

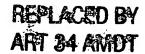
antigen-2 (EBNA2), human T-cell leukemia virus-1 (HTLV-1), HTLV-1 tax, Herpesvirus saimiri (HVS), mutant p53, myc, c-jun, c-ras, c-Ha-ras, h-ras, v-src, c-fgr, myb, c-myc, n-myc, and Mdm2.

- 5 64. The cell preparation of claim 58, wherein the cell types naturally secret one or more biologically active molecules.
  - 65. The cell preparation of claim 58, wherein the cell types are genetically engineered to secrete an exogenous level of at least one angiogenic factor, at least one growth/cytokine factor, or a combination of at least one angiogenic factor and at least one growth/cytokine factor.
  - 66. The cell preparation of claim 65, wherein the secretion of the at least one angiogenic factor, the at least one growth/cytokine factor, or the combination of at least one angiogenic growth and at least one growth/cytokine factor is controlled by gene switching.
  - 67. The cell preparation of claim 65, wherein the at least one angiogenic factor, the at least one growth/cytokine factor, or the combination of at least one angiogenic growth and at least one growth/cytokine factor is constitutively secreted.
  - 68. The cell preparation of claim 58, wherein the second component optionally further comprises a cryoprotectant.
- 25 69. The cell preparation of claim 68, wherein the cryoprotectant is selected from the group consisting of a 10% glycerol solution, a 15% glycerol solution, and a 15% glycerol and 5% human serum albumin solution
  - 70. A method of using the cell preparation of claim 58, the method comprising
  - a) providing the first component;
    - b) providing the second component;



- c) combining the first and second components to form a cell preparation suitable for tissue regeneration; and
- d) administering the cell preparation to a wound site.
- 5 71. The method of claim 70, wherein the cell types are differentiated fibroblasts and keratinocytes.
  - 72. The method of claim 70, wherein the cell preparation is in the form of a paste.
- 10 73. The method of claim 70, wherein the biologically active molecule is at least one angiogenic factor, at least one growth/cytokine factor, or a combination of at least one angiogenic factor and at least one growth/cytokine factor.
- 74. The method of claim 70, wherein the cell types are mitotically inactivated by
   administration of mitomycin C or other chemically-based mitotic inhibitors,
   irradiation with γ-Rays, irradiation with X-Rays, or irradiation with UV light.
- 75. The method of claim 70, wherein the cell types are immortalized using at least one gene/polypeptide selected from the group consisting of the 12S and 13S products of the adenovirus E1A genes, hTERT, SV40 small T antigen, SV40 large T antigen, papilloma viruses E6 and E7, the Epstein-Barr Virus (EBV), Epstein-Barr nuclear antigen-2 (EBNA2), human T-cell leukemia virus-1 (HTLV-1), HTLV-1 tax, Herpesvirus saimiri (HVS), mutant p53, myc, c-jun, c-ras, c-Ha-ras, h-ras, v-src, c-fgr, myb, c-myc, n-myc, and Mdm2.
- 25
- 76. The method of claim 70, wherein the cell types naturally secrete one or more biologically active molecules.
- 77. The method of claim 70, wherein the cell types are genetically engineered to secrete an exogenous level of at least one angiogenic factor, at least one growth/cytokine factor, or a combination of at least one angiogenic factor and at least one growth/cytokine factor.

15



- 78. The method of claim 77, wherein the secretion of the at least one angiogenic factor, the at least one growth/cytokine factor, or the combination of at least one angiogenic growth and at least one growth/cytokine factor is controlled by gene switching.
- 79. The method of claim 77, wherein the at least one angiogenic factor, the at least one growth/cytokine factor, or the combination of at least one angiogenic growth and at least one growth/cytokine factor is constitutively secreted.
- 10 80. The method of claim 70, wherein the second component optionally further comprises a cryoprotectant.
  - 81. The method of claim 80, wherein the cryoprotectant is selected from the group consisting of a 10% glycerol solution, a 15% glycerol solution, and a 15% glycerol and 5% human serum albumin solution
  - 82. The method of claim 70, wherein the first and second components are topically administered to the wound site on the patient.
- 20 83. The method of claim 70, wherein the first and second components are sprayed onto the wound site on the patient.
  - 84. The method of claim 83, wherein the first and second components are combined on the wound site.
  - 85. The method of claim 83, wherein the first and second components are combined before reaching the wound site.
- 86. The kit of claim 1, wherein the first component and the second component are cryopreserved prior to shipping and subsequently thawed prior to use.



- 87. The kit of claim 86, wherein the first and second components are each contained in a separate vial having a removable screw cap, wherein the vial is sterile and is made of a material resistant to low temperatures and wherein the removable lid can be replaced with a spray pump following thawing of the first and second components prior to use.
- 88. The kit of claim 87, wherein the spray pump delivers a volume of approximately 130 μl per spray.
- The kit of claim 86, wherein the material resistant to low temperatures is selected from the group consisting of glass, polypropylene, polyethylene, and ethylene vinyl acetate (EVA).
- 90. The kit of claim 87, wherein the vials are sealed within a pouch or container prior to cryopreservation, wherein the pouch or container is fabricated of a material capable of withstanding temperatures ranging from -80°C to -196°C and wherein the pouch or container protects the first and second components from contamination during cryopreservation and subsequent thawing.
- 20. 91. The kit of claim 90, wherein the pouch or container is waterproof and has a high barrier performance.